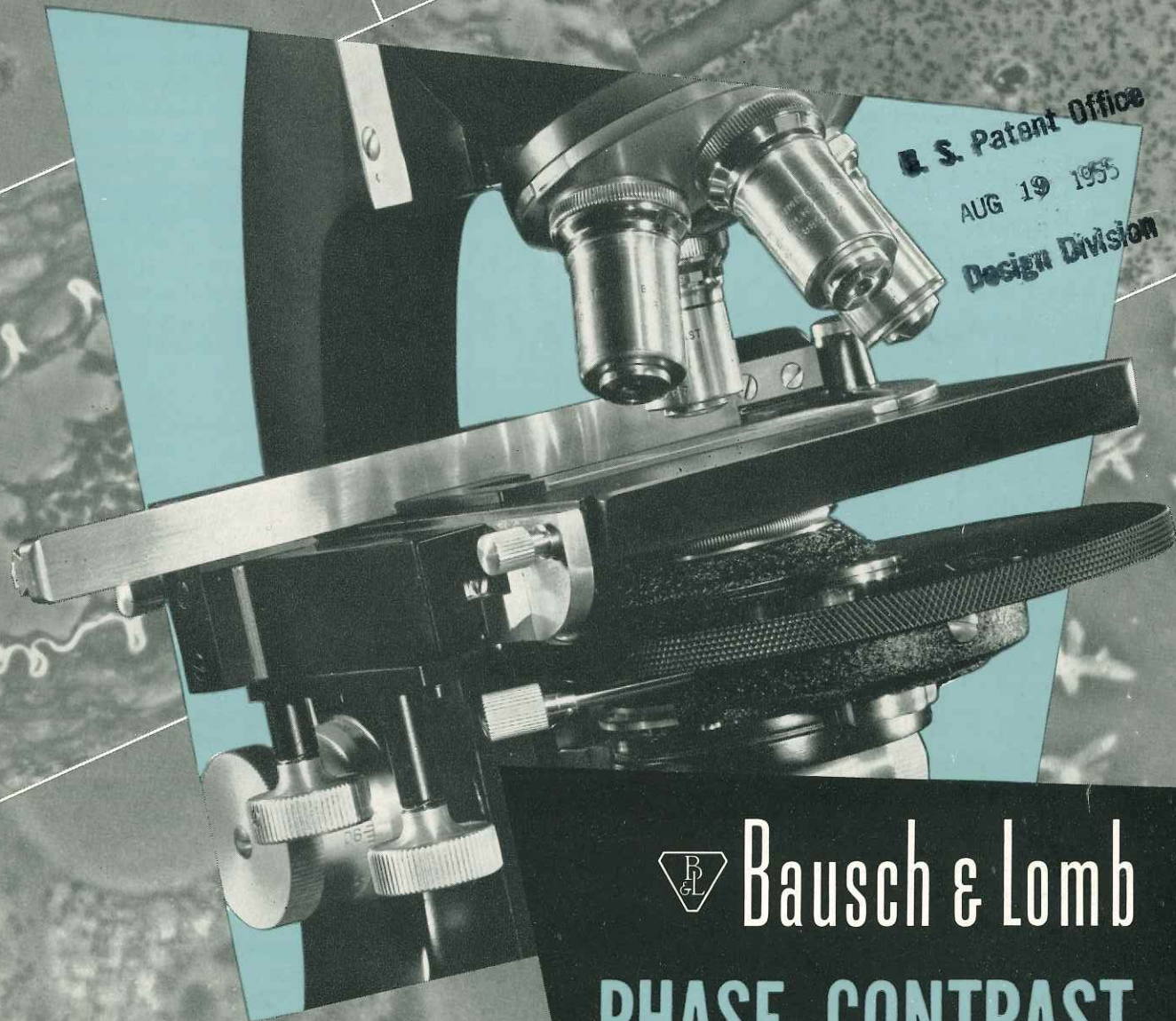


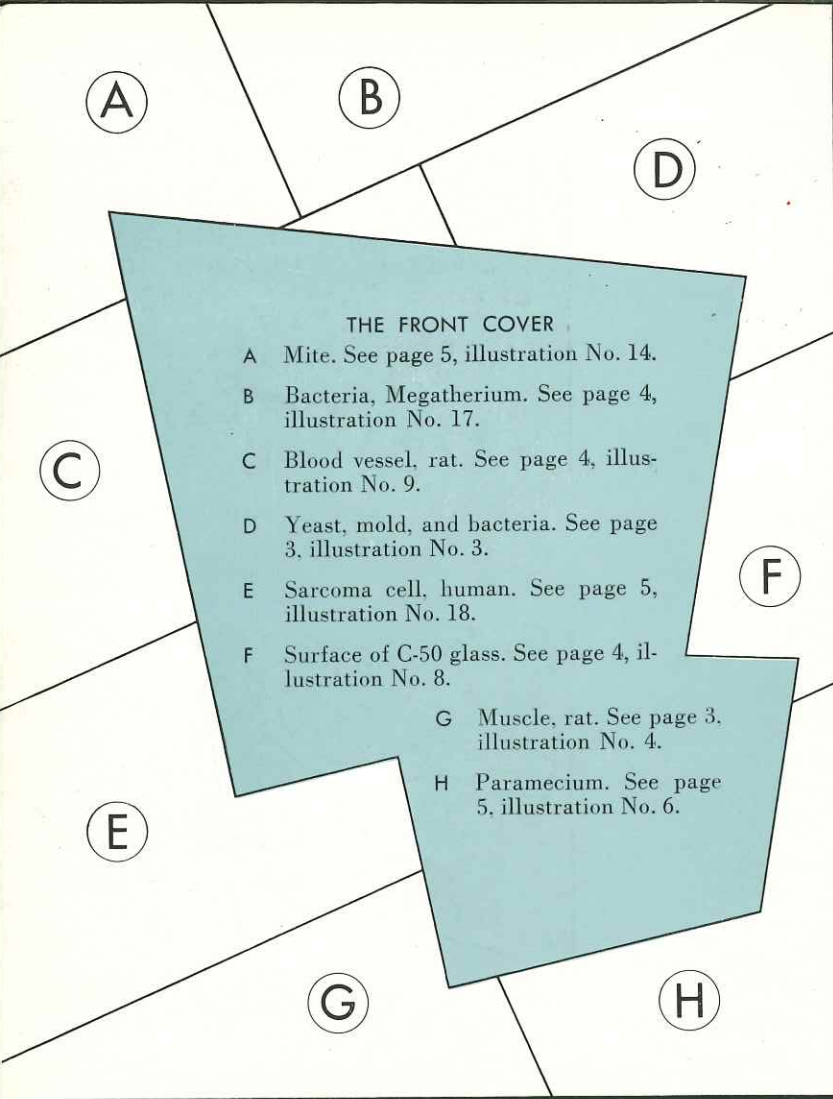
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U. S. Patent Office
AUG 19 1955
Design Division



Bausch & Lomb
PHASE CONTRAST
ACCESSORIES



Practical

THE MICROSCOPIST has always had difficulty in observing specimens that were colorless and differed little in optical path (refractive index $\times$ thickness) from their surroundings. In an effort to improve this condition the mineralogist and chemist make use of mounting media of lower or higher indices, giving contrast to the preparation with reference to its background.

Bright-field and dark-field illumination are employed, or polarized light in the case of double refracting material. Although these methods greatly improve the over-all contrast between the preparation and the mounting medium, they fail for the most part to produce contrast within the specimen.

The biologist, in studying living cells, is much more limited in his choice of methods. The mounting media must necessarily be body fluids or other isotonic solutions that will not cause shrinking or dissolution of the cell protoplasm or permit its escape from the cell.

Unfortunately, all liquids having these characteristics are of low index, slightly above water, and so close to the index of most cell constituents that there is little contrast between the specimen and the mounting medium.

The preferred method for living cells has been the use of non-toxic dyes commonly termed "vital dyes". This method of staining is often difficult and time consuming, and the number of structures of protoplasm that will stain by "vital dyes" is very limited.

Where dyes are of little or no value, the biologist has resorted to the out-of-focus image. For example, if a living epithelium cell from the mouth is examined in saliva at slightly above a good focus, structures such as the nucleus and certain cytoplasmic granules of higher index appear bright. Conversely, cell structures that have a lower index appear dark.

Phase microscopy allows us to examine such differences in index with all structures in the cell at exact focus. With dark contrast phase objectives,

The Theory of Phase Contrast Microscopy

In this catalog you will find a rather complete explanation of the Phase Contrast Method. While a clear conception of this principle is unnecessary for the successful use of the phase accessories, yet the inquiring mind seeks at least a working knowledge of this modern technique.

For those who do not care to go deeply into the scientific explanation there is included a highly condensed definition.

Practical Applications of Phase Contrast

A full use of Phase Contrast Accessories amply justifies their initial cost. Broadly separating themselves into the general classifications of biology, chemistry, mineralogy and counting, applications of this comparatively new technique are particularly noteworthy in the examination of living cells.

In the following pages are given many examples of practical applications, which, in turn, will suggest many, many more.

Uses of Phase Contrast

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objects of greater optical path than their surroundings appear dark, while those of less optical path present a lighter aspect. The effect obtained is as if the cell were stained with a dye coloring each constituent with an intensity proportional to its optical path.

Many papers have been published on phase contrast microscopy and further research will reveal many new applications. It is being continually demonstrated that it is applicable to the examination of all transparent material showing slight differences in optical path encountered by the bacteriologist, parasitologist, protozoologist, embryologist, histologist, pathologist, botanist, chemist, and mineralogist.

Briefly stated, a few of these applications classified by subject follow:

BIOLOGY

1. The applications of phase contrast in biological science are very many, especially with reference to the living cell. Bacteria in body fluids or cultures usually have a higher index than the liquid and appear dark on a lighter background with dark contrast phase objectives. Spores and flagella, difficult to demonstrate even in fixed dyed preparations, can often be observed in living bacteria. Spirochetes of the mouth and other body fluids usually examined in the living state by means of special dark-field condensers, or after impregnation with silver stains, are successfully applicable to the phase method.

Nuclei, cell granules, and cell walls of yeast, mold, and fungi can be observed with much greater contrast, the results dependent somewhat on the age and metabolism of the cell.

Paramecium, amoeba and other protozoa can be studied under phase contrast as living cells with detailed visibility of such structures as nuclei, food and water vacuoles, cilia, and flagella. Smears, stretched, teased or macerated preparations, and tissue cultures can be examined without the use of dyes, or, in some cases, to advantage in combination with vital or supravital dyes, or

Feulgen's stain.

2. Phase contrast microscopy is becoming increasingly valuable for the examination of fixed, stained microtome sections for increasing the detail of structures that are difficult to stain, or which, when dyed, show little differentiation. Stained or impregnated specimens, illustrations 5a-5b, that appear faded under bright-field illumination often show good contrast and detail under phase contrast. In many cases, provided mounting media of the correct index are employed, staining methods are unnecessary for microtome sections. Phase microscopy is especially valuable for the study of many neurological preparations which have been usually demonstrated in microtome sections by difficult impregnation methods.

3. Microincinerated zoological and botanical specimens have been very difficult to interpret. While the polarizing microscope has been of some value in this field of biology, phase contrast microscopy, with and without polarized light, is an important additional tool.

CHEMISTRY AND MINERALOGY

1. Thin sections of rock containing minerals of similar refractive indices such as quartz and feldspars are satisfactorily examined.

2. The refractive index of minerals and chemicals by the immersion method can be more accurately determined.

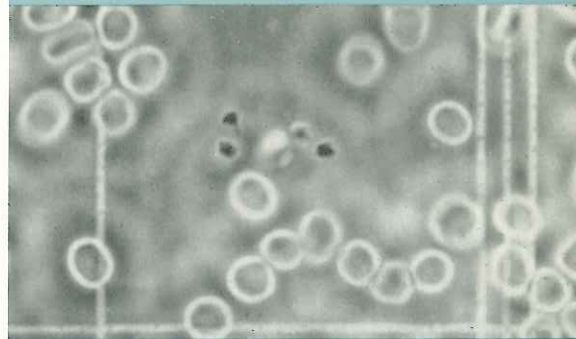
3. Inclusions in crystals or particles on the surface having a slightly lower or higher optical path than that of the crystal can be readily observed. The degree of purity of a specimen can be readily determined.

4. Phase contrast microscopy can be combined with polarized light by using either a polarizer or analyzer. Double refracting minerals or chemicals that are not readily visible at one optical orientation can be reoriented by use of a circular stage. Reorientation appreciably increases their visibility.

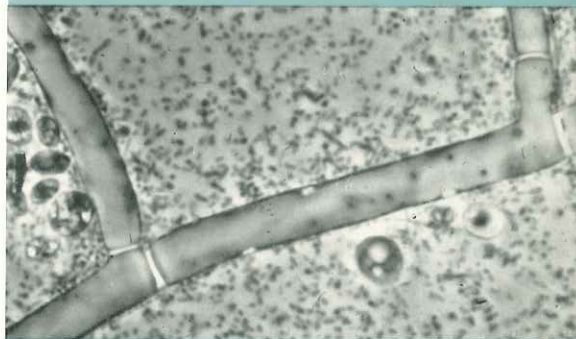
For example, a small crystal of sodium nitrate in a 1.587 index liquid, oriented for that index, would be some-



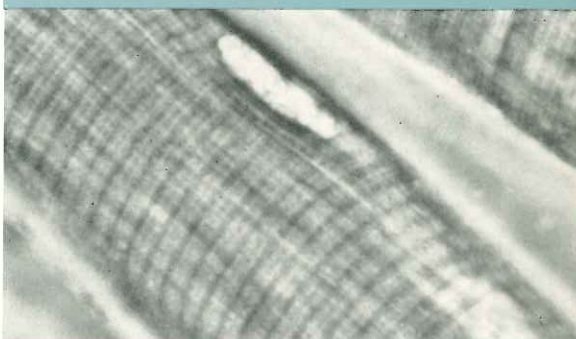
1. SPIROCHETES OF VINCENT'S ANGINA
Motile, living spirochetes in saliva. Original magnification, 970X. Phase contrast.



2. BLOOD PLATELETS
Original magnification, 350X. Phase contrast.



3. YEAST, MOLD, AND BACTERIA
From agar plate. Original magnification, 430X. Phase contrast.



4. MUSCLE, RAT
Showing nucleus. Original magnification, 1200X. Phase contrast.

what difficult to see. However, if the crystal be rotated 90 degrees on the circular stage for the 1.336 index, the difference in index between the specimen and the liquid is such as to give very good contrast. Provided the specimen and liquid are close in index for yellow light, and differ greatly in dispersion, "optical dispersion staining" results.

COUNTING

1. Particles are necessarily often counted in liquids close to the index of the particle, such as white blood cells in 0.1 N hydrochloric acid. The contrast between the cells and liquid is so small that the "above-focus" method previously mentioned is often used to bring out nuclear structure.

With phase contrast the nucleus and cytoplasm are so well differentiated from the background that a differential count can be obtained without resort to the stained smear method.

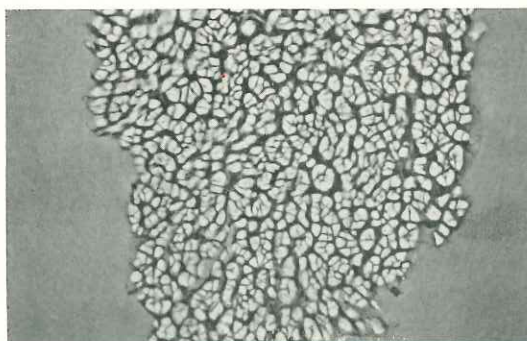
Other applications are the counting of red blood cells, blood platelets, bacteria, yeast, mold, plankton, and dust particles. The rulings on glass counting chambers are much more visible. They appear bright on a gray background somewhat similar to that obtained with dark-field illumination.

2. With non-phase contrast objectives, particle aggregates often appear as one particle. With phase contrast, aggregates can very often be separated into individual particles, whose boundaries or other differentiating characteristics are much better shown.

MISCELLANEOUS

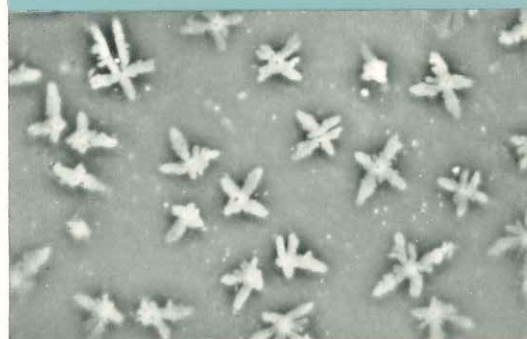
1. Surface structure of glass (*see illustration 8*) and other transparent materials, such as fine polishing marks and non-homogeneities, are more readily observed with phase contrast than by the usual methods. In the case of opaque material or metals, replicas of such surfaces may be first prepared and then examined with phase contrast objectives.

2. Textiles and paper products are applicable to phase contrast microscopy, both from the standpoint of easier identification and for dyeing, finishing and pigment problems. This method is of value for locating voids and fiber inclusions, as well as in the study of resin and other filler penetration.



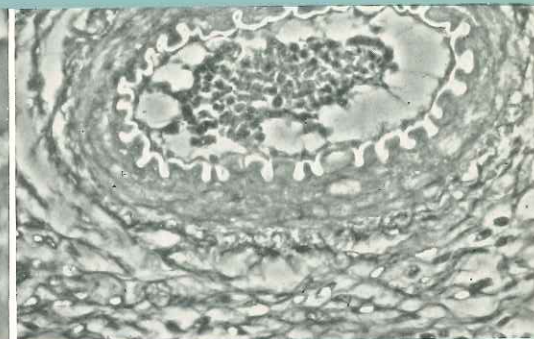
5a, 5b. LIGAMENT NUCHAE, COW

Original magnification, 300X. 5a (left), in 1.528 index liquid. 5b (right), in 1.508 index liquid. Both phase contrast.



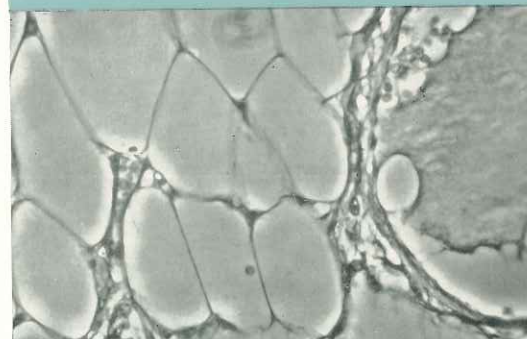
8. SURFACE OF C-50 GLASS

After exposure to humid atmosphere for 52 days. Original magnification, 970X. Phase contrast.



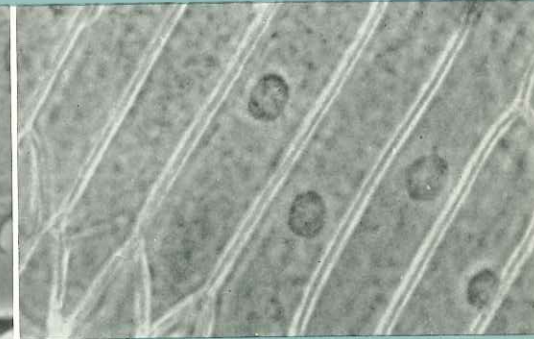
9. BLOOD VESSEL, RAT

Cross section with elastic fibre showing white. Original magnification, 300X. Phase contrast.



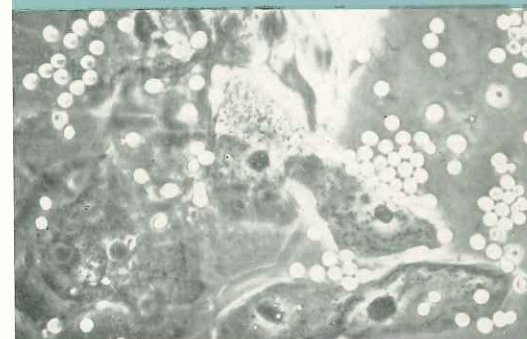
12. ADIPOSE TISSUE, RAT

Fat removed by dehydration, leaving protoplasmic envelopes and nuclei. Original magnification, 300X. Phase contrast.



13. ONION SKIN

Original magnification, 210X. Phase contrast.



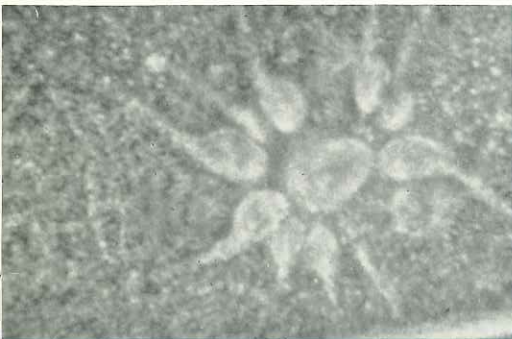
16. URINE

Sediment from centrifuged specimen. Original magnification, 300X. Phase contrast.



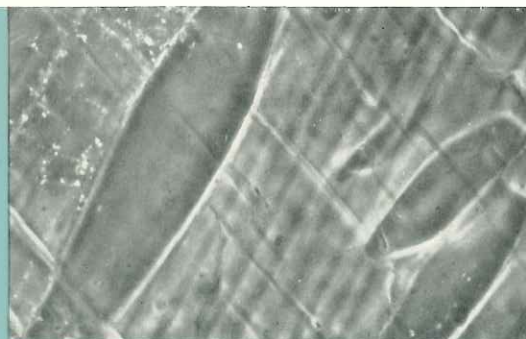
17. BACTERIA, MEGATHERIUM

Living bacteria, spores showing white. Original magnification, 970X. Phase contrast.



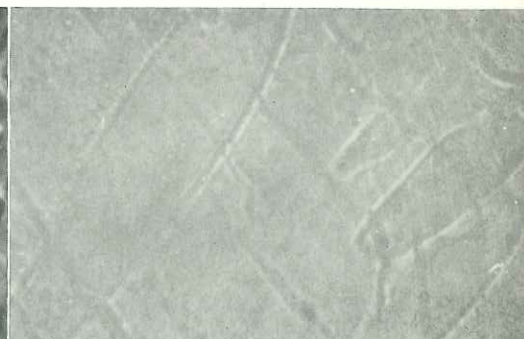
6. PARAMECIUM

Showing contractile vacuole expanded. Original magnification, 430X. Phase contrast.



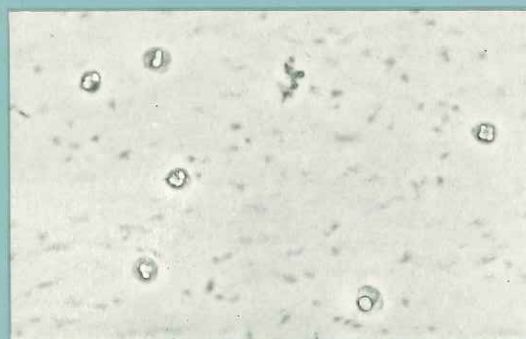
7a, 7b. FELDSPAR

Cleavage, perthite and slight alteration in thin section. Original magnification, 430X. 7a (left), phase contrast. 7b (right), bright field, reduced aperture.



10. SPERMATOZOON, HUMAN

Head, neck, and tail plainly seen. Original magnification, 970X. Phase contrast.



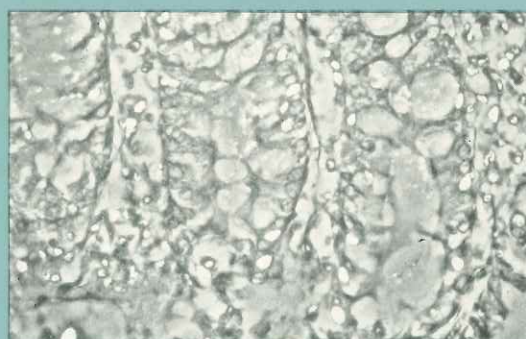
11a, 11b. WHITE BLOOD CELLS

Original magnification, 210X. 11a (left), phase contrast. 11b (right), bright field.



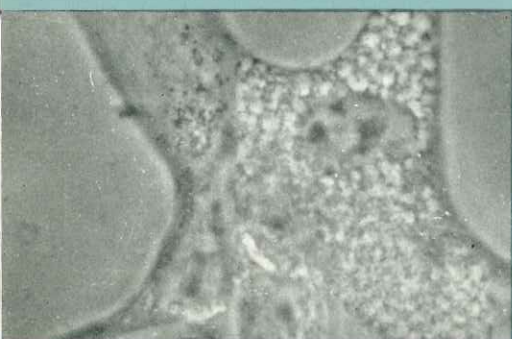
14. MITE

Unstained, whole mount of *Demodex folliculorum* from dog. Balsam mounted. Original magnification, 300X. Phase contrast.



15a, 15b. STOMACH, RAT

Unstained, paraffin section of mucous membrane. Original magnification, 300X. 15a (left), phase contrast. 15b (right), bright field, reduced aperture.



18. SARCOMA CELL, HUMAN

In tissue culture. Reticular cell. Original magnification, 430X. Phase contrast.



19, 19b. LEPTOSPIRA ICTEROHAEMORRHAGIAE

Stained by Fontana's method. Although specimen has faded, satisfactory detail is observed with phase contrast. Original magnification, 300X. 19a (left), phase contrast. 19b (right), bright field.



PHASE CONTRAST ACCESSORIES

Turret-type Abbe Condenser

• Simplicity, convenience and efficiency are the outstanding characteristics of the Bausch & Lomb Turret-type Abbe Condenser Phase Contrast Accessories. Users enthusiastically endorse the Bausch & Lomb policy of designing this equipment so that its successful use requires few variables.

SIMPLIFIED EQUIPMENT

The complete equipment consists of: 1) An assembly of condenser, with iris diaphragm, in stationary stage, and rotatable changer containing four diaphragm stops with annular rings, one for each objective, and one free aperture for bright field use; 2) Four objectives with phase altering patterns in the rear focal planes; 3) Auxiliary telescope; 4) Green filter; 5) Shillaber's oil.

Any B&L laboratory microscope hav-

ing a rack and pinion substage can be employed. No fuss or bother is entailed in mounting the changer.

OBJECTIVES

The Phase Contrast Objectives are fitted with standard Society threads and screw into the nosepiece exactly the same as do the conventional type. Available in 16mm, 8mm, 4mm, and 1.8mm, they give magnifications of 10 $\times$, 21 $\times$, 43 $\times$, and 97 $\times$ (oil immersion).

TELESCOPE

For pre-use centration of the condenser diaphragm image and the phase altering pattern of the objectives an auxiliary telescope is supplied. This adjustment for concentricity of the two rings is quickly effected by means of two knurled thumb screws in the turret-type condenser.

ONLY THREE OPERATIONS

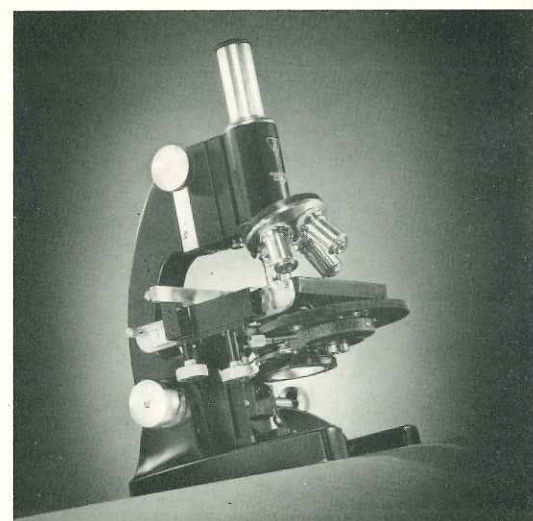
Full operating instructions are furnished with each set of accessories. Briefly, there are but three simple, quickly performed steps: 1) Turn the proper objective into the optical path by rotating the nosepiece; 2) Rotate the turret until the corresponding annular stop is in the optical axis (there is a window opening in the condenser stage through which numbers indicative of the magnifications of the corresponding objectives appear); 3) Center the condenser diaphragm image and the phase altering pattern by using the auxiliary telescope in place of the eyepiece.

SEPARATE STANDS AVAILABLE

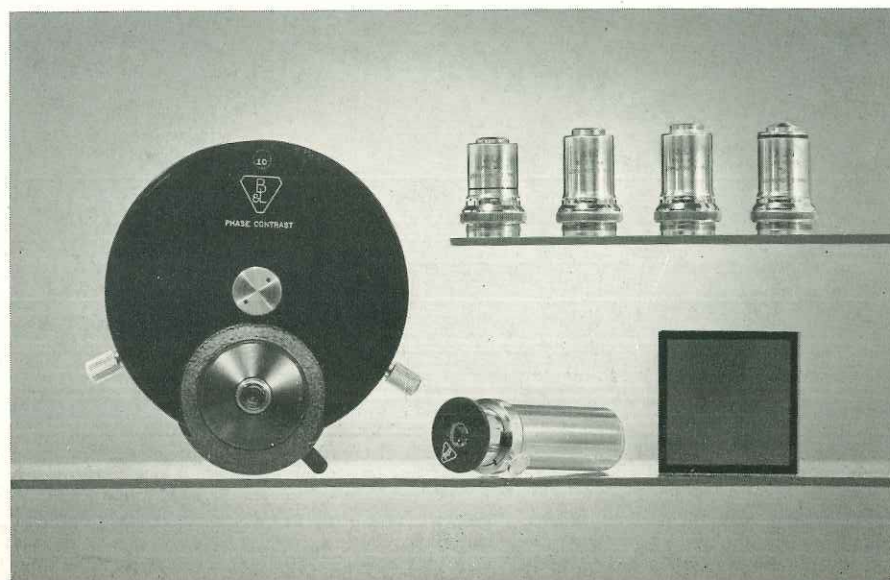
Many users have found that it is necessary to keep a microscope for phase contrast work exclusively. Therefore, two types of stands, monocular and binocular, are listed on page 10. On these stands can be placed the B&L Phase Accessories, giving the purchaser the widest possible choice.

SPECIFICATIONS

Description	Catalog No.
Rotatable, turret-type changer, with four annular stops and one free opening; iris diaphragm; Abbe 1.25 N.A. condenser; centering telescope; and green filter; one dispensing oil bottle; in case.	31-58-78-02
PHASE CONTRAST OBJECTIVES Select one or more	
97 $\times$, 1.8mm, 1.25 N.A., oil immersion	31-10-70-02
43 $\times$, 4mm, 0.65 N.A.	31-10-99-01
21 $\times$, 8mm, 0.50 N.A.	31-10-97-01
10 $\times$, 16mm, 0.25 N.A.	31-10-92-01
For basic items under catalog No. 31-58-78-02 and one of each of the four objectives, 31-10-99-01, 31-10-97-01, 31-10-92-01, 31-10-70-02 order.	31-58-78-01



Above: The Turret-type Condenser Phase Contrast Accessories on Bausch & Lomb Model FBR Monocular Tube Microscope, and (at right) on Model TBR with interchangeable binocular body.



Left: The complete equipment less immersion oil and case.



PHASE CONTRAST ACCESSORIES

Individual Annular Stop Type L.W.D. Condenser

• Answering an insistent demand for a condenser for phase contrast examination of tissue cultures in flasks, and counting objects in thick chambers, such as the Bausch & Lomb Hemacytometer, the Individual Annular Stop L.W.D. (Long Working Distance) Condenser Phase Contrast Accessories have been designed. Utilizing the same "positive" or "dark" contrast as do the Turret-type Condenser Accessories, the L.W.D. group follows the proven Bausch & Lomb interpretation of Zernike's theory. Results from the use of these accessories have been highly satisfactory.

FOR RESEARCH OR LABORATORY INSTRUMENTS

The condenser has a long working dis-

tance of from 8mm to 10mm in air, allowing examination of cultures under natural conditions. This condenser is regularly supplied with a dovetail slide for fitting to any B&L research type microscope with complete centering substage. For use on laboratory type instruments the slide can be unscrewed from the condenser and replaced by an iris diaphragm.

OBJECTIVES

Objectives are the same as are employed in the Turret-type accessories, 16mm, 8mm, 4mm, and 1.8mm—magnifications being 10×, 21×, 43× and 97×, respectively.

ANNULAR STOPS

Individual annular stops are available

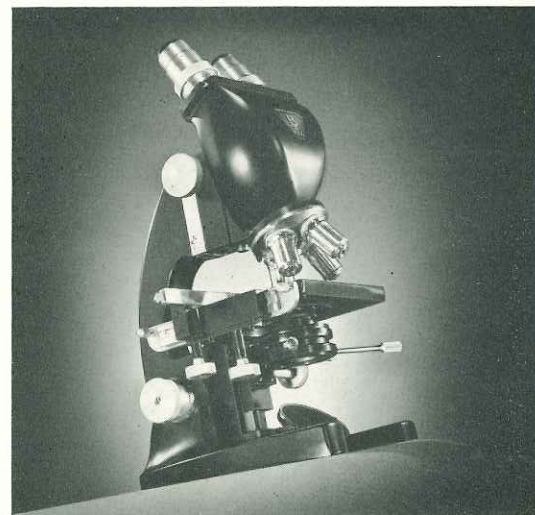
which correspond to the various objectives. When stops and objectives are ordered together, the annular rings in the stops are accurately centered to the phase patterns in the objectives, and after the original adjustment require practically no more centering even with frequent change of objectives and stops.

SIMPLIFIED EQUIPMENT

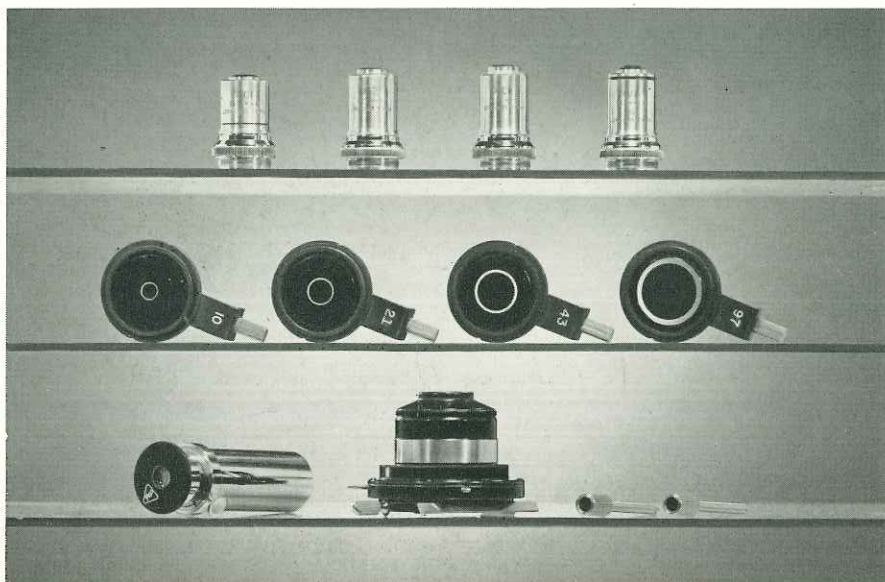
Complete L.W.D. equipment consists of the condenser with two centering key-heads, centering telescope, green filter, dispensing oil bottle, in leatherette-covered carrying case, plus four objectives and four corresponding annular stops. Only such objectives with stops as may be necessary need be ordered, but the balance of the equipment is required. When advisable to assign a special microscope to phase work, choice of a binocular or monocular stand is explained on page 10.

SPECIFICATIONS

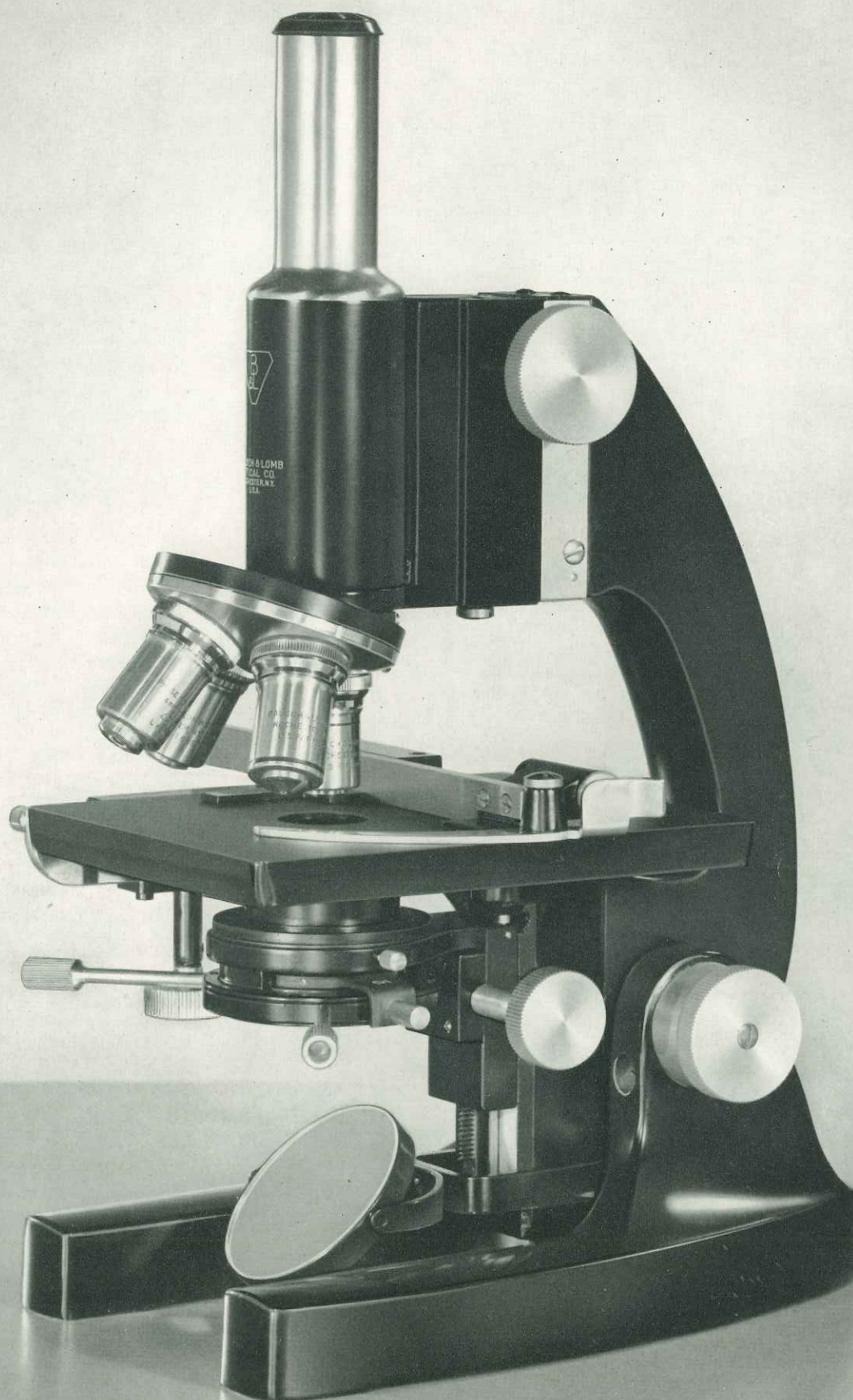
Description	Catalog No.
Long Working Distance Condenser, mounted on plate for research type substage*; centering telescope; and green filter; one dispensing oil bottle; in case *Specify if iris diaphragm is wanted in place of research substage plate.	31-58-88-02
PHASE CONTRAST OBJECTIVES	
97×, 1.8mm, 1.25 N.A., oil immersion	31-10-70-02
43×, 4mm, 0.65 N.A.	31-10-99-01
21×, 8mm, 0.50 N.A.	31-10-97-01
10×, 16mm, 0.25 N.A.	31-10-92-01
INDIVIDUAL ANNULAR STOPS	
Specify corresponding stop for each objective ordered.	
For objective	
97×, 1.8mm, 1.25 N.A., oil immersion	31-58-94
43×, 4mm, 0.65 N.A.	31-58-93
21×, 8mm, 0.50 N.A.	31-58-92
10×, 16mm, 0.25 N.A.	31-58-91

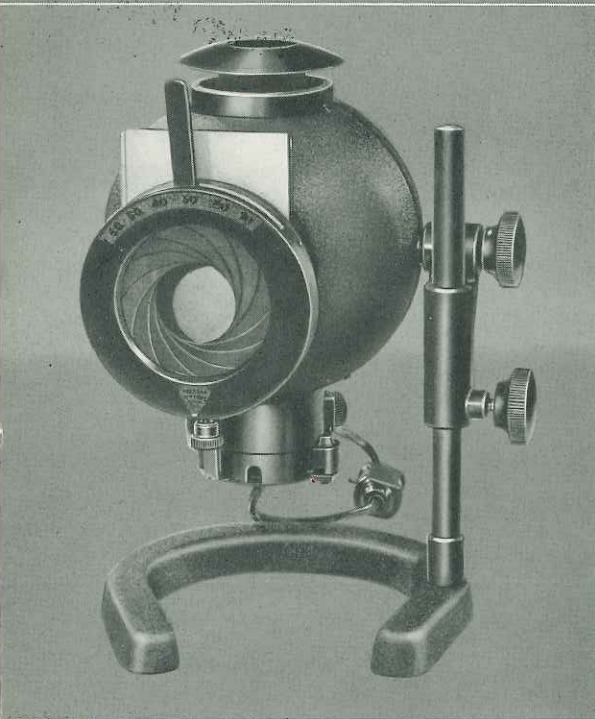
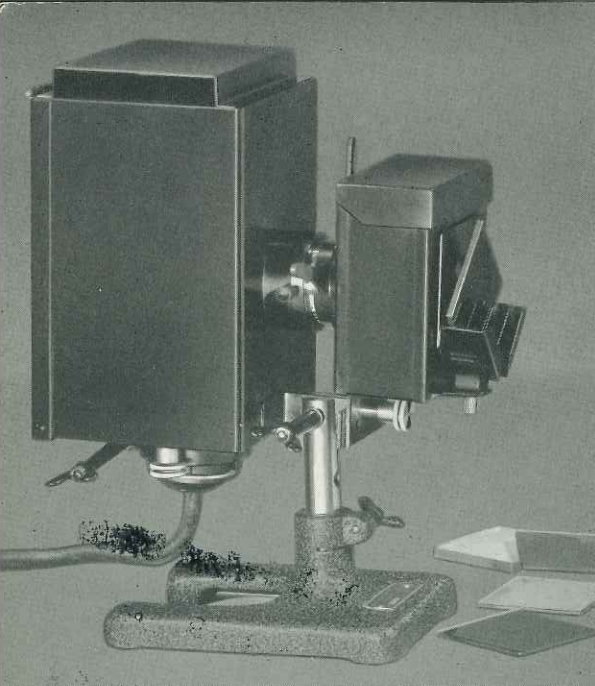


Above: The Individual Annular Stop L.W.D. Phase Contrast Accessories on Bausch & Lomb Model TBR Microscope with interchangeable binocular body and (at right) on Model FBR Monocular Tube Microscope.



Left: The complete group less filter, case and immersion oil. Basic items are grouped, for ordering, while the objectives and their corresponding annular stops are to be ordered individually.





Illuminators, Replacements, Stands

• Illumination for Phase Contrast Microscopy is no more complicated a problem than that presented by bright-field work. The main requirement, of course, is that the annular ring be evenly and adequately lighted. Bausch & Lomb offers two illuminators which are well suited to this type of work — the Research and the Spherical, both with 6-volt ribbon filament lamps.

RESEARCH ILLUMINATOR

For either type of Phase Contrast Accessories this instrument is highly satisfactory — the full aperture of the substage condenser is utilized and there is an abundance of light on the specimen. A set of four neutral and one daylight filters is included.

The green filter which is included as a part of each type of B&L Phase Contrast Accessories is preferable for the reason that the phase shift is calibrated in monochromatic light of that wavelength. Therefore, a realization of the greatest benefits in the phase contrast method depends on the green filter's use. This filter fits the filter holder of the Research Illuminator.

Upper: Bausch & Lomb Research Illuminator with water cell and filter holder, and four neutral and one ground glass filters. Lower: The Spherical Illuminator. A special green filter should be ordered, as the one regularly supplied with the Phase Contrast Accessories will not fit the slot in this Illuminator. Transformers for 115-v., 60-cycle, A.C., are included but not shown in these illustrations.

SPHERICAL ILLUMINATOR

Some users prefer the Spherical Illuminator with spherical reflector, and aspheric focusable condenser, and it does a commendable job in furnishing critical illumination for phase contrast. This Bausch & Lomb instrument can also be used for Koehler illumination of the condenser annulus.

A ground glass, as well as a blue filter, is included and, while the 50mm square green filter regularly supplied with the B&L Phase Contrast Accessories will not fit the filter slot of this Spherical Illuminator, there is one listed in the specifications which should be ordered for this instrument.

MICROSCOPE STANDS

In order to enable the purchaser to exercise the widest latitude in the choice of his Phase Contrast Microscope, Bausch & Lomb supplies two microscope stands with eyepieces but less objectives and substage condensers.

The binocular stand, TBR, is shown on page 7 carrying the Turret-type Condenser Accessories, while the monocular type, FBR, appears on page 9, fitted with the Individual Annular Stop L.W.D. Accessories. However, either stand will accommodate either group of accessories.

SPECIFICATIONS

FBR Stand, with vertical monocular tube, 39mm diameter; with 10× Huygenian eyepiece; rack and pinion coarse adjustment; ball-bearing and roller low position fine adjustment; rack and pinion substage with full 360° ring mount for carrying Bausch & Lomb phase contrast condensers; ball-bearing, ball-stop, precentered, quadruple nosepiece; ungraduated, mechanical stage with vacuum controlled spring slide finger; plano-concave mirror in tuning-fork, quick-on bracket; alcohol- and reagent-resistant black finish; in hardwood carrying case with lock and key; Catalog No. 31-20-30

TBR Stand, interchangeable, inclined binocular body with parallel eyepiece tubes; with paired 10× Huygenian eyepieces; rack and pinion coarse adjustment; ball-bearing and roller low position fine adjustment; rack and pinion substage with full 360° ring mount for carrying Bausch & Lomb phase contrast condensers; ball-bearing, ball-stop, precentered, quadruple nosepiece; ungraduated, mechanical stage with vacuum controlled spring slide finger; plano-concave mirror in tuning-fork, quick-on bracket; alcohol- and reagent-resistant black finish; in hardwood carrying case with lock and key; Catalog No. 31-20-62

SPECIFICATIONS

Description	Catalog No.
ILLUMINATORS	
Spherical Microscope Illuminator with aspheric condenser; blue and ground glass filters; 108-w., 6-v., ribbon filament lamp; iris diaphragm; cord; plug; and transformer for 115-v., 60-cycle, A. C.	31-33-78-54
Green Filter for use in Spherical Illuminator with phase contrast accessories...	31-34-54
Research Microscope Illuminator with corrected condenser; 108-w., 6-v., ribbon filament prefocused base lamp; combination filter and water cell holder; four neutral and one daylight glass filters, 50mm square; and transformer for 115-v., 60-cycle, A. C.; and Pyrex water cell	31-33-85-11
PHASE CONTRAST ACCESSORIES REPLACEMENTS	
Wratten Filter, green	42-47-61
Centering Telescope	31-50-78
Shillaber's Oil, No. 595, in Dispensing Bottle.	31-50-95

Unstained, colorless, transparent specimens under standard bright field illumination are generally lacking in contrast. In order to obtain contrast so that structural detail may be recognized and studied, time consuming methods of preparation have been necessary. These have often resulted in physical or chemical changes in the specimen, especially in the case of living biological material.

The phase contrast method of microscopy overcomes this difficulty. It permits the examination of living, unstained material without resort to the usual staining procedure. The method is also applicable to fixed and dry specimens including microtome sections. Provided mounting media of correct index are employed, selective differentiation of structure in fixed specimens is often possible without the use of stains.



THE PRINCIPLE OF PHASE CONTRAST

U. S. Patent Office

AUG 19 1955

Design Division

RESOLVING POWER OF THE MICROSCOPE

In describing phase contrast microscopy, it is necessary to review, briefly, the theory of image formation in the microscope itself. The microscope is essentially a magnifier, and as such, its general function is to make visible detail which is too small for the unaided eye to see. Magnifying power, however, is not the most important characteristic if the observer is to see detail. More important than magnifying power is resolving power, which is dependent upon the numerical aperture of the microscope objective.

DIFFICULTIES WITH TRANSPARENT MATERIALS

Granted sufficient resolving power, considerable difficulty is encountered in the use of conventional microscopy for the examination of fine detail in transparent material. This problem commonly occurs in the examination of many living cells, tissue, and small living organisms which are usually transparent and are mounted in transparent media of almost identical optical properties. The solution of this problem, up to now, has been commonly reached by one of three methods. Stained material may be examined by bright field. Unstained materials may be examined by either or both dark field and polarized light.

These methods, however, have their limitations. Staining techniques often-times distort the specimen; dark field sometimes exaggerates the contrast of the specimen and the background so that the gradations possessed by the specimen structure are not shown in their proper proportions. Surface layers, rather than internal details, are generally revealed. Polarized light is limited to the examination of anisotropic material.

PHASE CONTRAST, THE ANSWER

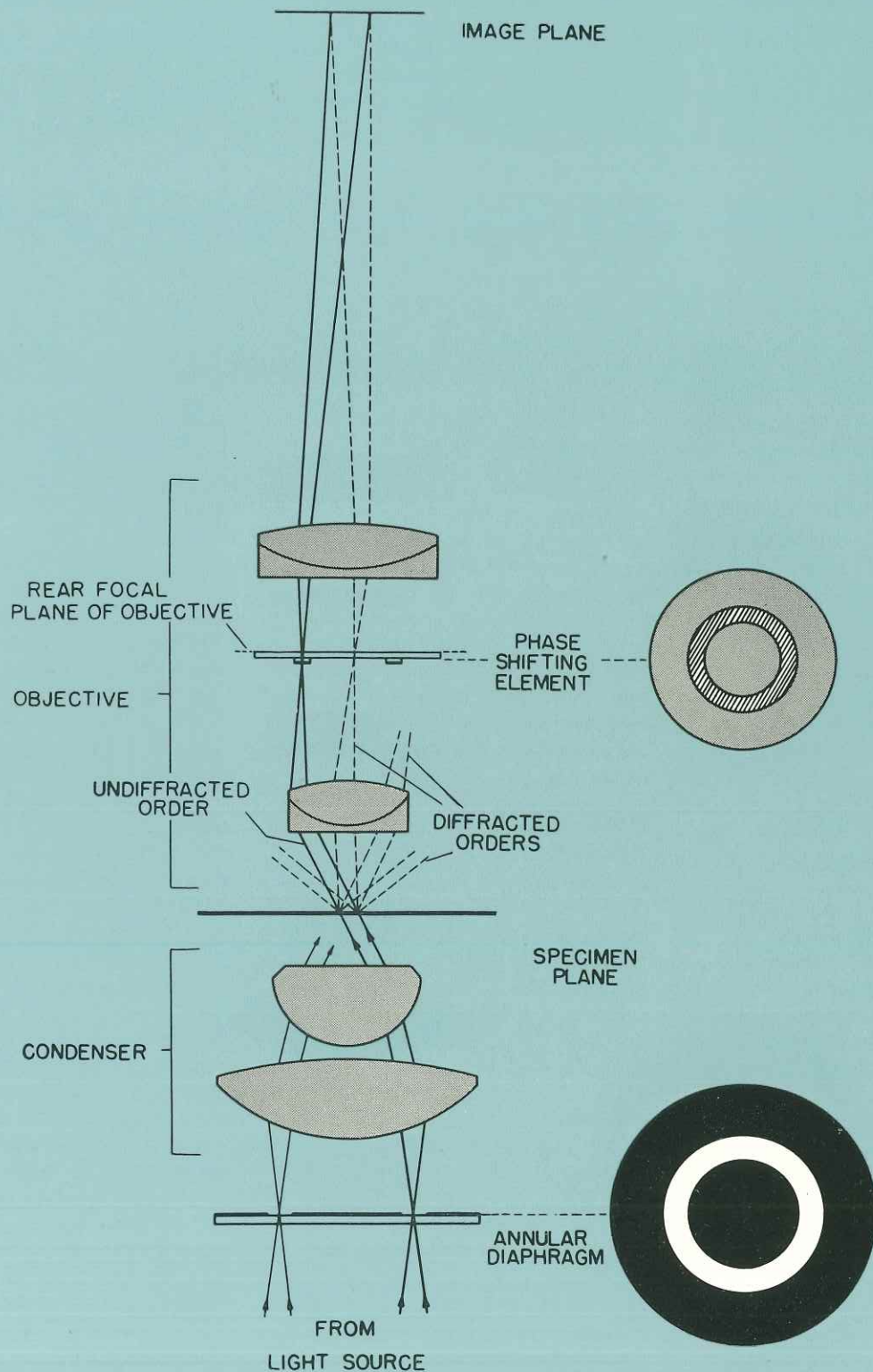
It might be generally said that the microscopist desires to study material in as near as possible to its natural state. A new method of illumination, incorporating the principles of phase contrast microscopy, first successfully demonstrated by F. Zernike in 1935, overcomes these difficulties, and is at least a large step toward the ultimate goal. Microscope image formation depends on the physical phenomenon of diffraction. When light falls upon an object having fine structure, the characteristic change brought about may be described as a breaking up of the beam into discrete bundles of rays diverging from the object detail over a wide angle. Diffraction thus occurs at the object plane, and the objective lens receives not only the direct light through the specimen but

all other diffracted orders of light falling within the angular limits of the objective aperture. If the source is one providing parallel light, all such bundles entering the objective are imaged at its rear focal plane where they may be observed as a series of maxima and minima diffraction spectra images. In this plane, these images act as secondary sources, and send out their own cones of light. Since these images have related phases in this plane, their effect in any other plane may be influenced, controlled, and measured through observable effects. Hence, since all of the light emanates from the same original source, the related spectra become visible through their interference effects wherever they overlap.

It is at this point, in the final image plane, that conditions of structure and phase, as they exist in the original specimen, are reproduced. If a change is introduced in the relative phases of the spectra appearing at the rear focal plane of the objective, the intensity in the final image will vary. Evidence of structure detail will be revealed as a consequence. In the rear focal plane of the objective, a phase difference is introduced in the phase contrast method of microscopy. In this manner, it is possible to suppress, intensify, and even reverse the contrast in the final image, depending upon the nature of the phase shift introduced.

Bausch & Lomb Phase Contrast Accessories

Light Path and Optical Elements



On these pages is a detailed explanation of the principle of phase contrast. To readers interested in the mechanics of this technique it will prove very illuminating. For others, primarily concerned with the end result, a condensed explanation is more in order.

Phase Contrast Microscopy is, essentially, a special method of controlled illumination, ideally suited for observing thin, transparent objects whose structural details vary only slightly in thickness and refractive index.

These slight variations are not sufficient in themselves to be visible in the bright-field microscope. They do, however, affect light passing through the specimen, causing slight irregularities in the wave fronts. Minor changes in the phase relation of the wave fronts result. Phase contrast equipment transforms these phase changes into corresponding variations of brightness, so that the structure is visible under conditions of enhanced contrast.

IN BRIEF

IMAGE FORMATION —PHASE CONTRAST

An annular aperture in the diaphragm, placed in the focal plane of the substage condenser, controls the illumination on the object. The aperture is imaged by the condenser and objective at the rear focal plane, or exit pupil, of the objective. In this plane a phase shifting element, or phase plate, is placed.

Light, shown by the solid lines and undeviated by the object structure, in passing through the phase altering pattern, acquires a one-quarter wavelength of green light advance over that diffracted by the object structure (broken lines) and passing through that region of the phase plate not covered by the altering pattern. The resultant interference effects of the two portions of light form the final image. Altered phase relations in the illuminating rays, induced by otherwise invisible elements in the specimen, are translated into brightness differences by the phase altering plate.

(The eyepiece is not shown in this diagram.)

PHASE ALTERING ANNULI

Phase altering patterns, commonly referred to as "phase plates," are expedients designed to effect a change in an optical path, or phase relation, between that part of the light entering the objective directly through the object structure, and that part which is diffracted by the structure and also enters the objective. Such phase altering patterns are made by the deposition of films of predetermined thicknesses by high vacuum thermal evaporation processes. These films may be applied to separate glass discs mounted in the objective, or applied directly to a lens surface, depending upon the objective design. Patterns, which have been found to be most practical and effective, are those of the annular type, which introduce a phase shift of one-quarter of a wavelength of green light.

An annular aperture diaphragm is necessary for all types of annular phase altering patterns. It is placed at the front focal plane of the substage condenser and, when illuminated, becomes an effective source at infinity with

respect to the object plane. Such an annular source illuminates the object with a hollow cone of light, and controls the obliquity of the light traversing the medium.

The shape of the illuminating aperture is important in the formation of diffraction images, since such images actually determine the nature of the final image through their interference effects, and are images not of the specimen itself, but of the illuminating aperture as influenced by the specimen structure.

For the most critical conditions of illumination, light is focused on the substage condenser annular diaphragm, using the principles of Koehler illumination, to fill it uniformly with light. The real image of the diaphragm is then reproduced through the condenser, object, and objective, at the rear focal plane of the objective.

This, then, satisfies the condition that the object may be considered to be illuminated by a number of point sources located at infinity. Also, at the rear focal plane are imaged the diffraction spectra produced by the object structure.

FORMATION OF PHASE DIFFERENCE

The phase altering annulus is placed in the rear focal plane of the objective and its size is such that it coincides with the image of the substage annulus. When the two annuli are exactly concentric and superimposed, direct transmitted light will pass through the annular region and will differ in phase by one-quarter of a wavelength from the light diffracted by the object and entering the objective. This light diffracted by the object passes through the remaining aperture area, which is not covered by the annulus.

Since the effect of the shift may be either to accelerate or retard the phase relation of the directly transmitted light, with respect to the diffracted portion, either positive or negative contrast may result. If the film thickness contributing to the phase shift is such that the directly transmitted light passing through the phase altering element is effectively accelerated over the diffracted light by one-quarter of a wavelength of light, the resulting contrast is referred to as "positive" or "dark" contrast, and the phase altering element is said to be "accelerating". Positive contrast shows regions of greater optical path in the specimen to be darker than the surrounding background.

"Negative" or "bright" contrast is produced by a retardation of the undiffracted light, regions of greater optical path in the specimen appearing bright against a darker background. The image is thus similar to a dark field image. While such an objective is easily produced, popular demand has been in favor of "dark" contrast, since the results obtained have greater similarity to the usual hematoxylin stained bright field image. An added benefit is greatly enhanced contrast and graded variations.

Since most microscopic specimens exhibit irregular structure, the diffraction spectra are not clearly defined, and the direct image of the illuminating source, only, is sharply defined. This direct light, in addition, is much more intense than the diffracted light.

METALLIC ABSORBING FILM

Since the direct light always completely participates in the image formation, its intensity would tend to overbalance that from the weaker, secondary diffraction maxima. The relative distribution of light between these two determines the amount of contrast available. To compensate for this unbalanced distribution of light, a metallic absorbing film is combined with the phase element to reduce the intensity of the direct light. This absorption tends to equalize the intensities of the two interfering beams and thus increases the sensitivity of contrast by permitting the weak diffracted orders to effect a proportionately greater influence. If the direct beam is not sufficiently dampened (inconel deposit on the phase annulus) to equalize its brightness with that of the diffracted beam, the two will interfere, but there will be an excess of light in the direct beam beyond that necessary for the interference process. That direct light will dilute image contrast. If it is excessive, regular bright field microscopy results. The diffracted beam would be drowned out. Similarly, if the diffracted beam is out of balance, the image will be devoid of contrast. The latter results if the inconel deposit be made opaque and the diffracted beam, only, proceeds to the image plane. It is, therefore, obvious that by varying the transmission of the annulus, there can be produced, at will, the complete range from no contrast all the way to a pseudo dark field. The best contrast somewhere in between is that giving a gradation of darkness for the various degrees of densities within the specimen. Bright field gives no contrast at all unless the specimen is thick or absorbs strongly, and dark field gives the extreme black and white contrast occluding everything in between.

TRANSMISSION COATING CAREFULLY CHOSEN

The choice of the balancing inconel transmission coating is therefore a delicate one, and it has been carefully studied. Assuming that the specimen is nearly transparent in bright-field illumina-

tion, the determination of the transmission is contained in the Bausch & Lomb objectives. If, however, the specimen absorbs strongly in certain areas, a different balance would have to be established. But, in that case, the specimen absorption in itself produces contrast. The phase accessories increase contrast in the nonabsorbing sections of the specimen, yielding a desired contrast balance.

Theoretically, monochromatic light of the same wavelength used for the original calibration, should be used when working with phase accessories, if the full phase shift effect is to be realized. It is for this reason that green filters are made available with the Bausch & Lomb Phase Contrast Accessories. The one-quarter wavelength phase shift produced by the B&L accessories is calculated in the green portion of the spectrum. Use of other wavelengths will not produce the same degree of phase shift. If the microscopist chooses to use white light, extinction by interference will not be complete for all colors, since the specimen detail does not exert the same phase altering power for all colors.

Phase Contrast Microscopy and Related Subjects

Reprints of Published Papers by Bausch & Lomb Personnel

Mounting Media for Phase Microscope Specimens. *Stain Tech.*, 24, 241, 1949.

Dispersion Staining with Phase Contrast Microscope Accessories. *The Microscopic Identification of Quartz*. Science, 110, 237, 1949.

Counting of Dust Particles by Phase Microscopy. *Arch. Indust. Hyg. Occup. Med.*, 6, 416, 1952.

The Counting of Blood Cells by Phase Microscopy. *Am. Jnl. of Clin. Path.*, 20, 1041, 1950.

Counting with the Phase Microscope. *The Ed. Focus.*, 22, 1951.

Copies of these papers can be had, without charge, by writing Bausch & Lomb Optical Co., Rochester 2, N. Y.

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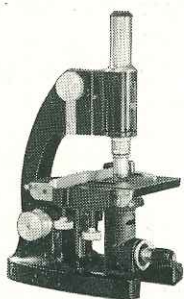
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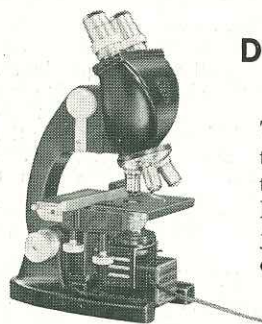
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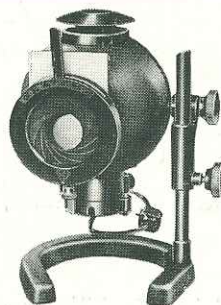
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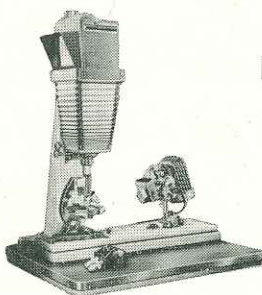
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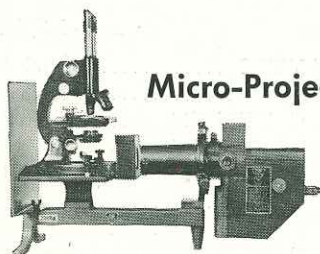
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